Normal and Pathologic Biochemistry of the Lung

by Aron B. Fisher*

The lung is an important metabolic organ. Glucose provides the major source of substrate. Oxidative pathways are active and are required to maintain normal tissue energy stores. Synthetic activity in the lung is extensive and related mainly to synthesis of dipalmitoyl lecithin and other components of the lung surfactant system. The lung plays an important role in the metabolism of xenobiotics and endogenous hormones leading to degradation as well as activation of important biologic properties.

Only recently has the lung been recognized as an important metabolic organ rather than just a tissue for passive gas exchange. A major reason for the delay in appreciation of the metabolic role of the lung is related to its structure and anatomic relationships. The lung, although filling most of the thoracic cavity, actually comprises only 1% of the body weight, and approximately 30% of that weight is due to contained blood. Further, the blood flow to the lung comprises the entire cardiac output making it the most richly perfused organ in the body. Because of the high blood flow in relation to metabolizing tissue mass, arteriovenous differences of most metabolites cannot be measured across the lung *in situ*. Consequently, it has been necessary to develop *in vitro* models for study of lung metabolism. One model that has been extensively used is the isolated perfused lung preparation. Perfusion of the lung with artificial media removes the red cells from the pulmonary capillaries and results in tissue with a completely white appearance. Additional models to study lung metabolism are tissue slices and preparations of subcellular organelles.

By use of these preparations, it has been possible to gain insights into lung metabolism, although our understanding is still far from complete. This paper will be a superficial survey of activity in this new field and will focus specifically on oxygen utilization and energy generation, lung surfactant and metabolism, and metabolism of hormones and xenobiotics.

Oxygen Uptake

Measurements with the isolated perfused lung preparation or lung slices have shown oxygen uptake in the range of 30-150 μl/min-g dry weight, depending on species and preparation (1). Therefore, lung tissue has significant O_2 consumption although values are low compared with the metabolically very active organs. For example, dog lung oxygen uptake per unit weight is only 10-20% of the oxygen uptake of canine heart, kidney, thyroid, and brain (1). The oxygen uptake of the lung is greater, however, than that of resting skeletal muscle, intestine, and many other metabolically less active tissues. Actually, the lung can be considered an average organ in terms of O_2 utilization, since its oxygen uptake represents approximately 1% of the total oxygen

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uptake of the organism, and this is approximately the contribution of the lung to total body weight. On the other hand, it should be noted that the lung represents a heterogeneous collection of cell types, and it is likely that some components of the lung, e.g., the type II granular pneumocytes, have considerably higher oxygen uptake than the mean for the whole lung.

Substrate Utilization by Lungs

What are the substrates utilized by the lung for its metabolic requirements? Although intact lungs and lung subcellular organelles can oxidize fatty acids (2, 3), glucose probably serves as the major oxidizable substrate under usual conditions. Glucose utilization by the isolated perfused lung under control conditions is approximately 40 μ mole/hr/g dry weight (4). In the presence of an inhibitor of oxidative metabolism, the rate of glucose utilization may double indicating a rather brisk Pasteur effect (5). A similar increase in glycolytic rate can be found during perfusion with an uncoupler of oxidative phosphorylation (D. Bassett and A. Fisher, unpublished observations). The fate of the carbons from glucose metabolized by an isolated lung preparation is shown in Figure 1. In this and many similar studies in the literature, approximately half of the glucose was converted to lactate and pyruvate. The "reason" for the high rate of produc-

tion of these three-carbon compounds has not been defined. One postulated explanation relates to the presence of numerous cells in the lung with relatively sparse mitochondria and, therefore, limited citric acid cycle activity. Additional possibilities include limited activity of mitochondrial H⁺ shuttle mechanisms or mitochondrial pyruvate dehydrogenase. In any case, the high lactate production under control conditions was probably not due to cellular hypoxia since the lung was being ventilated with 95% O_2, the perfusate L/P ratio was within a normal range (i.e., 5-10), and the lung responded briskly to inhibitors of oxidative metabolism with change in redox state (5). Approximately one fourth of the glucose carbons utilized by the perfused lungs are oxidized to CO_2. In our experiments, about 25% of this CO_2 is derived from the pentose shunt pathway and the remainder from mitochondrial oxidation (D. Bassett and A. Fisher, unpublished observations). There are also active pathways for incorporation of glucose carbons into tissue components including proteins, nucleic acids, polysaccharides (chiefly glycogen), and other unidentified components. Finally, a small but significant fraction of glucose carbons is used for synthesis of lipids, including the fatty acid as well as glyceride-glycerol moieties.

Energy Stores of the Lung

The next question to explore is whether oxidative metabolism is required in order to maintain normal energy stores of the lung tissue. Insight into this problem can be obtained by measurement of changes in lung tissue adenine nucleotide content during inhibition of oxidative metabolism, or uncoupling of oxidative phosphorylation. During control perfusion, ATP content of the lung per unit weight is comparable to values observed in other aerobic tissues and the ATP/ADP ratio is approximately 8.5 (Fig. 2). During inhibition of oxidative metabolism with carbon monoxide ventilation, there is a marked decrease in the lung ATP content as well as decreases in the ATP/ADP ratio. During perfusion with dinitrophenol, an uncoupler of oxidative phosphorylation, lung ATP content and ATP/ADP ratio also decrease, although the change is less marked than with CO ventilation. These results indicate that oxidative metabolism is required in order to maintain normal lung tissue energy stores and suggest that energy-dependent metabolic processes will be depressed subsequent to inhibition of oxidative metabolism.
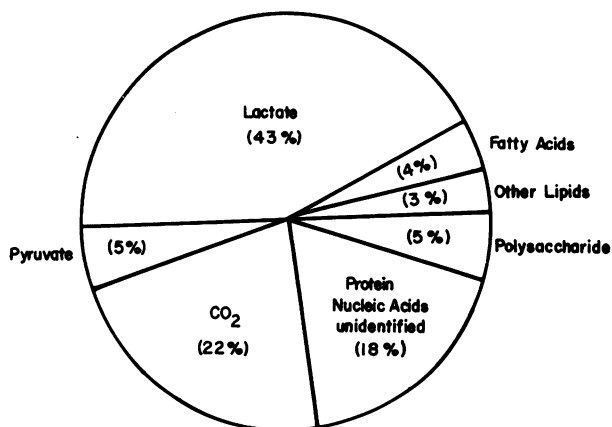

FIGURE 1. Recovery of carbon atoms derived from glucose in various products of metabolism during perfusion of the isolated rat lung with 5.5mM [U-^{14}C] glucose in Krebs-Ringer bicarbonate solution, pH 7.4, containing 3% bovine serum albumin. Lactate and pyruvate were recovered in the perfusate, $^{14}CO_2$ was recovered in the perfusate plus ventilatory gas, and the other components were recovered in tissue extracts. The data are represented as % of total recovery.
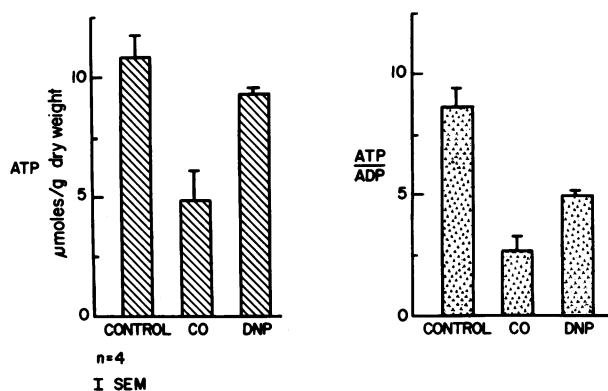
FIGURE 2. ATP content and ATP/ADP in isolated perfused rat lungs under control conditions and during ventilation with 95% CO_2:5% CO_2 (CO) or perfusion with $0.8mM$ dinitrophenol (DNP). At the end of 60-90 min of perfusion, lungs were rapidly frozen at the temperature of liquid N_2, extracted with cold ethanolic perchloric acid, and assayed for adenine nucleotide content by enzyme methods (5).

Energy-Dependent Lung Functions

What are the metabolic processes in the lung that are energy-dependent? Certainly the lung has no physiologic process that requires large expenditures of energy such as occurs with cardiac muscle contraction, renal transport, or maintenance of ionic gradients in nerve tissue. Energy utilization, however, is required for functioning of several lung systems. For example, lung clearance depends on bronchial ciliary activity and phagocytosis by alveolar macrophages, both of which are energy dependent. During anoxia, there is cessation of ciliary beating (6) and inhibition of particle phagocytosis (7). Secretion by bronchial glands and constriction of tracheobronchial smooth muscle are other processes that presumably are energy-dependent. Synthesis of dipalmitoyl lecithin, a major component of the lung surfactant system, requires a supply of ATP (8). The actual secretion of surfactant is also probably an energy-requiring process analogous to cellular secretion elsewhere. Finally, energy is required for cell transport processes, including those that maintain the cellular internal milieu and provide substrate as well as specialized transport functions such as the active uptake of 5-HT (see below). Recognition of these energy-dependent physiologic parameters suggest that some lung functions might be impaired in the absence of oxidative metabolism.

Lung Surfactant

The most dramatic metabolic function of the lung and the one that has been studied to the greatest extent relates to the synthesis of lung surfactant. The lung surfactant is contained in the extracellular alveolar lining layer that coats the epithelial surface of the lung alveoli. The presumed physiologic function of the surfactant system is to maintain the surface tension at the interface between the alveolar surface and the air spaces at low levels and thereby promote alveolar stability. The extracellular material can be obtained for study by alveolar lavage with saline or other physiologic solution. The material so obtained contains approximately 75% phospholipids, 15% neutral lipids, and 10% protein (9). Dipalmitoyl lecithin (dipalmitoyl phosphatidylcholine) accounts for approximately 40% of the total solids in the surfactant fraction. Synthetic dipalmitoyl lecithin (DPL) manifests similar surface active properties to the crude surfactant fraction so that the physiologic properties of the surfactant are thought to be related chiefly to the presence of DPL. An additional 25% of the surfactant material is comprised of lecithins containing unsaturated fatty acids. The physiologic role of this fraction of the surfactant is not known but may be important as an aid to spreading or otherwise influencing dispersion of the dipalmitoyl lecithin fraction. The role of the protein in surfactant is also incompletely understood. Current evidence suggests that the protein is specific to the lung and that surfactant is secreted as a lipoprotein complex.

Surfactant Synthesis and Turnover

What is the source of surfactant and what are the pathways involved in its production? It now seems clear that surfactant is synthesized in the lung and subsequently secreted onto the alveolar surface. Most studies that have investigated surfactant production have focused on pathways of dipalmitoyl lecithin synthesis although, as noted above, DPL is only one component of the surfactant fraction. These studies have shown that DPL is synthesized chiefly by the classical pathway involving phosphatidic acid (formed from glycerol-3-phosphate and palmitate) and CDP-choline (formed from CTP and choline). Considerable effort is currently being directed to determine the factors that are important in control of this pathway in the lung.

Interesting insight into the overall importance of surfactant synthesis to lung economy has been

obtained by analysis of the relative proportions of the various fatty acids in lung tissue. Compared with other organs, such as the brain, liver, and kidney, lungs from a variety of species have an unusually high proportion of palmitate in relation to other saturated or unsaturated fatty acids (10). Therefore, lung tissue appears to be geared towards utilization of palmitate and subsequent synthesis of dipalmitoyl lecithin. Extraction of free fatty acids from the blood supply is one likely source of the palmitate that is required for DPL synthesis. Significant uptake of palmitate into the lung was noted within 5 min after injection of tritiated palmitate into rats (11). By autoradiography, most of the label was found in type II alveolar cells (11), which therefore, seem to be most active in accumulating fatty acids from the circulation. In addition to uptake from the free fatty acid fraction, palmitate may also be derived from circulating lipoproteins. This source is possible, since lung endothelium is rich in heparin-activated lipoprotein lipase (12), which can hydrolyze lipoproteins into constituent fatty acids thereby creating a potential fatty acid reservoir for lung uptake. In addition to uptake of fatty acids from the vascular space, fatty acids can be synthesized intracellularly *de novo* from two-carbon precursors derived from glucose (see above), although this pathway is probably not sufficient to supply totally the fatty acid requirements of the lung. The factors controlling the relative role for each of these sources of palmitate have not been determined.

The metabolic requirements for palmitate are a function of the turnover rate for this moiety in the synthesis of surfactant. Following injection of C^{14} palmitate *in vivo*, radioactivity accumulates in lung tissue and reaches peak values in about 1 hr, followed by a gradual decline of radioactivity with half-time of approximately 12 hr (13). The appearance of radioactivity in alveolar lavage material is delayed relative to lung tissue (peak values are reached after approximately 6 hr), but declines at a similar rate as observed in lung tissue (13). These results suggest movement of palmitate from tissue to alveolar space with a relatively rapid turnover. Turnover time for other portions of surfactant material may be slightly longer, suggesting that some components are recycled, but the general conclusion is that synthetic activity for all components of the surfactant system is an active process requiring continuing metabolic activity.

Based on experiments of the type summarized above, the current concept is that surfactant synthesis is a major metabolic activity of the lung. Substrates for surfactant synthesis are transferred from the circulation or alveolar space to type II epithelial cells where the lipid and protein components are synthesized, presumably in the endoplasmic reticulum and Golgi organelles. These components are then packaged in lamellar bodies for storage and subsequently released onto the alveolar surface. Considerable work remains in order to define factors controlling synthesis, release, and turnover of surfactant.

Disorders of Surfactant Synthesis

Shortly after appreciation of the importance of surfactant in respiratory physiology, surfactant deficiency was found in lungs of infants with respiratory distress syndrome (RDS). Investigations into possible mechanisms have resulted in increased understanding of the development and maturation of the surfactant system. One key finding is that the lung surfactant system matures relatively late in the course of fetal development. In the monkey (gestation period 170 days), significant amounts of lecithin are not present in the alveolar lining material until gestation is approximately 80% complete (14). Appearance of lecithin on the alveolar surface correlates with a sharp increase of lecithin in the lung hemogenate and the presence of lamellar bodies in the type II alveolar cells. Since respiratory distress syndrome in the newborn is associated with prematurity, the concept has arisen that RDS represents birth of the fetus before complete maturation of the lung surfactant system. Recently, several groups have investigated potential mechanisms to accelerate the normal rate of maturation of the surfactant system. One promising method is administration of adrenocorticosteroid hormones which do appear to accelerate maturation and protect infants delivered prematurely against development of RDA (15). Several of the enzymes in the pathways of phospholipid synthesis undergo induction with steroid treatment which might account for the accelerated maturation (10). This area is under active investigation at the moment and promises great potential benefit in terms of the prevention of a common cause of infant mortality.

Metabolism of Hormones and Xenobiotics

The discussion up to this point has been concerned with intrinsic metabolism of the lung in order to maintain its structure and function.

Another important role for the lung that has recently been extensively investigated is concerned with the metabolism of xenobiotics and endogenous hormones. Through these pathways, the lungs are able to exert a major influence on bodily homeostasis. As one example of hormone metabolism, lungs rapidly clear serotonin (5-HT) from blood or other media perfusing the pulmonary circulation (17, 18). This amine can be taken up against a concentration gradient by a mechanism that shows saturation kinetics, is dependent on external sodium, and can be blocked by specific competitive inhibitors, inhibitors of oxidative metabolism, and by ouabain (Fig. 3). After uptake, serotonin is metabolized by a monoamine oxidase (MAO) to 5-hydroxy-indoleacetic acid. The rate of metabolism does not significantly influence the rate of uptake. These characteristics suggest that uptake of serotonin by the lung occurs by active transport and that transport is the rate limiting step in serotonin clearance. Autoradiographic studies have demonstrated that the transport process occurs almost

exclusively into the pulmonary endothelium (19). The mechanism for amine transport shows specificity, since histamine, another vasoactive compound, is not taken up nor metabolized by lung to any significant extent. As another example of specificity, norepinephrine is taken up and metabolized whereas epinephrine is not.

Uptake and metabolism is only one mechanism by which the lung transforms vasoactive compounds. An additional pattern is operative with respect to conversion of angiotensin I to angiotensin II (resulting in formation of a potent vasoactive compound) or hydrolysis of peptide bonds in brandykinin (resulting in a loss of biological activity). The responsible peptidase enzyme (converting enzyme) is either the same for both reactions or represents a group of closely related enzymes. Converting enzyme activity is present on the luminal surface of the pulmonary endothelium (20), so that hydrolysis occurs at the membrane surface and active transport into the cell is not required. A more complex relationship can be seen with several of the prostaglandins since the lung is an important site for uptake and metabolism as well as synthesis, storage, and release (21).

In addition to contact with endogenous agents, the lung is exposed to a wide variety of potentially toxic substances delivered either through inhalation or via the circulation. A major pathway for detoxification of foreign components such as drugs is through hydroxylation in the liver by cytochrome P-450-linked reactions. Recently, this pathway has also been demonstrated in isolated lung microsomes. These organelles from lung contain, on a weight basis, approximately 25% of the cytochrome P-450 activity of microsomes isolated from liver (22). More recently, we have demonstrated cytochrome P-450-linked activity in the isolated perfused rabbit and rat lung (23). The reaction studied in these experiments was the demethylation and hydroxylation of p-nitroanisole to p-nitrophenol which requires NADPH, molecular oxygen, and cytochrome P-450. Production of p-nitrophenol during infusion of p-nitroanisole was indicated by increased optical density (Fig. 4). Production could be reversibly blocked by ventilation with 80% carbon monoxide due to binding between CO and cytochrome P-450. The significance of this reaction in the lung has not been fully explored but may be important in the local detoxification of drugs, possibly explaining vagaries of drug activity on the lung. It has also been suggested that this pathway may be responsible for the local conver-
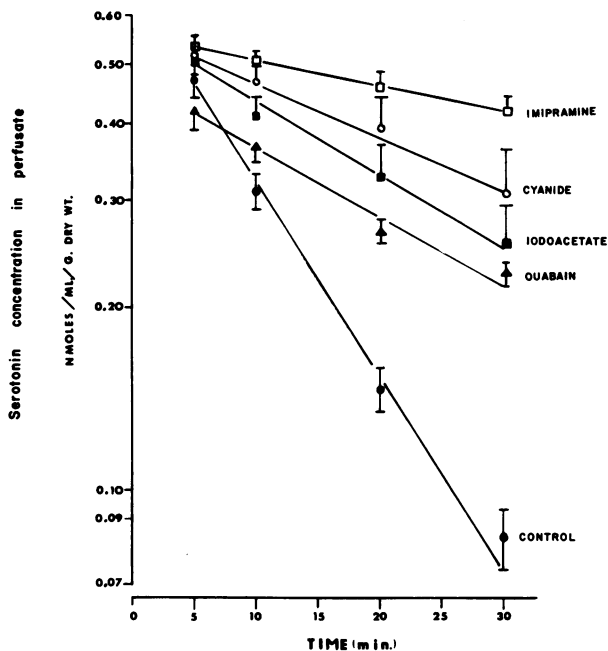

FIGURE 3. Disappearance of serotonin from the perfusate during perfusion of the isolated guinea pig lung. The concentration of serotonin in perfusate is plotted as a logarithmic function of time. Note the marked depression of serotonin uptake in the presence of $0.1mM$ imipramine (a competitive inhibitor of amine transport), $1mM$ KCN (an inhibitor of oxidative metabolism), $1mM$ sodium iodoacetate (an inhibitor of glycolysis), and $0.01mM$ ouabain (an inhibitor of Na$^+$–K$^+$-activated ATPase).
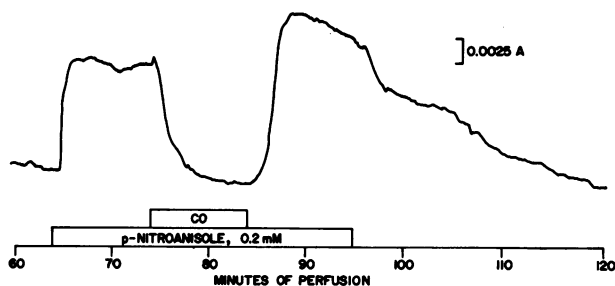
FIGURE 4. Demonstration of cytochrome P-450 activity in the isolated perfused rabbit lung. Lungs were perfused with a "once-through" system. The effluent was sampled continuously, and optical density (A) was monitored at 436 nm with a flowthrough cuvette. The start of p-nitroanisole infusion is indicated at the bottom of the figure. The increase in optical density indicates production of p-nitrophenol via a cytochrome P-450-linked reaction. The reaction is reversibly inhibited by ventilation of the lung with 75% CO:20% O_2: 5% CO_2 (indicated by CO).

sion of nontoxic compounds into carcinogenic agents.

Response of Lung to Oxidants

Is this role of the lung in the metabolism of vasoactive compounds and xenobiotics altered in the presence of lung disease? It seems likely that alteration of some of these important metabolic functions does occur, although investigations are in the preliminary stages. One area of extreme interest related to lung pathophysiology involves the response of the lung to oxidants, including the pollutants ozone and nitrogen dioxide as well as therapeutically administered oxygen in high concentrations. As an interesting metabolic correlation, activity of several enzymes, specifically those concerned with the pentose shunt pathway and dismutation of the superoxide anion, may be involved in protection of the lung against oxidant damage (24). Recently, we have found that serotonin uptake by the isolated perfused lung is diminished by exposure to oxygen (25), and we have interpreted these changes as a manifestation of early pulmonary oxygen toxicity. In normal rats exposed to 100% oxygen at one atmosphere, approximately 20% depression of serotonin clearance was noted after 18 hr of exposure. This toxic effect of O_2 was greatly magnified in animals maintained on a vitamin E-deficient diet for 4-6 weeks. In these vitamin E-deficient animals, serotonin uptake was depressed approximately 50% after 12 hr of oxygen exposure. To put these findings in perspective, pulmonary functional alterations and electron microscopic

changes are generally not observed in normal rats until after approximately 24-48 hr of exposure. Therefore, measurement of lung metabolic function, in this case, serotonin uptake, may provide an early and sensitive index of pulmonary toxicity.

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